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ASSOCIATIONS BETWEEN BREAST CANCER AND DIETARY AND PLASMA LEVELS OF CAROTENOIDS AND VITAMIN A[1,2,3]

(Running title: Breast cancer and carotenoids and retinol)

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Abstract

A case-control study of breast cancer was conducted in Buffalo, New York. Participants completed a food frequency questionnaire and donated a fasting blood sample before definitive workup for breast masses. Dietary and plasma levels of carotenoids and retinol for 83 women found to have breast cancer (cases) were compared to those of 113 women found to be free of breast cancer (controls). Low levels of plasma beta-carotene were associated with increased risk of breast cancer ($p=0.02$). There was no overall association between retinol and breast cancer, but a positive relationship between retinol and breast cancer was present in the subgroup with low beta-carotene values. No statistically significant associations were observed in the dietary data. These results suggest that dietary practices associated with lower plasma beta-carotene values are associated with increased risk of breast cancer and that plasma indicators of nutrients may be more useful than dietary indicators of nutrient status in studies of limited sample size.

Key Words: Carotenoids, Vitamin A, Breast Cancer, Diet, Plasma, Benign Breast Disease, Epidemiology

Introduction

The roles of vitamin A and carotenes in carcinogenesis have been investigated in a large number of experimental systems (1). Supplemental retinyl acetate in animal diets may cause either lower (2-8) or higher (9) incidence of mammary tumors, whereas supplemental beta-carotene in the diet has been shown to inhibit mammary tumors (10,11) and transplantable tumors (12).

Epidemiological studies have indicated a negative association between plasma retinol and risk of cancer of all sites (13,14) with only suggestive evidence for this association in breast cancer (15). Two studies with dietary data (16,17) and three studies with plasma data (18-20) showed no association between breast cancer and retinol, whereas one study using plasma (16) showed a positive association. Two other dietary studies (21,22) indicated a negative association between breast cancer and total vitamin A intake with contributions from both preformed and precursor (carotenes) vitamin A.

A negative association has been observed between breast cancer and estimates of dietary intake of carotenes (17) and vegetables (23). Although results from two plasma studies (16,19) showed no association between breast cancer and total carotenoids or beta-carotene, respectively, results from two other plasma studies (18,24) reported a nonsignificant negative association between breast cancer and beta-carotene.

Overall, previous studies indicate either no association or a direct association between breast cancer and retinol, and no association or a negative association between carotenes and breast cancer. Some studies

were not specifically designed to investigate breast cancer and suffered from inadequate power (18-20), whereas others did not control for important breast cancer risk factors (19-21,24) or plasma lipids (18,20, 24). These limitations may have diminished the ability to observe the effects of nutrients on cancer risk.

This case-control study was designed to assess the independent and interactive effects of preformed vitamin A and carotenoids, in the plasma and in the diet, controlling for other risk factors and other nutrients.

Methods

Between September 1985 and September 1986, study subjects were enrolled from the breast clinic at Roswell Park Memorial Institute, and from the offices of two private surgeons in Buffalo, New York. Eligible patients were women 30 to 80 years of age who were being evaluated for a breast mass, but who had no previous history of cancer. All participants had lived in upstate New York or Pennsylvania for at least one year. After subjects were scheduled for a diagnostic biopsy, informed consent was obtained and they were given a questionnaire and directions for the blood collection procedure. Thirty-four patients who were referred to the specialists because of a suspicious mammogram or lump but were determined not to have masses requiring biopsy were included in the control group.

Patients were classified into one of three groups based on their pathology reports from the breast biopsies; cases (n=83), controls (n=113) or excluded (n=40). Cases were women found to have breast cancer (IDC9=174). The 113 controls were those 79 women who were found to have benign lesions on biopsy, plus the 34 women who did not require a biopsy.

The 40 patients with biopsy reports that described lesions thought to be at increased risk of breast cancer (predominantly of an atypical hyperplastic nature) were excluded from the control group.

The self-administered questionnaire requested information about the subjects' dietary practices and medical history. A food-frequency table with 5 frequency categories (never, rarely, 1-3x/month, 1-4x/week and 5-7x/week) contained a list of 30 food items that previous analysis (25) indicated would account for 90% of the variance associated with estimated vitamin A intake. Standard portion sizes were assumed to convert the frequency data into estimates of nutrient intake.

Fasting blood samples were drawn from subjects before their biopsies or on a designated day for the 34 patients without biopsies. The samples were centrifuged, plasma was preserved in sodium ascorbate (10 mg/ml plasma) and frozen at -80^{o}C until the end of the study. Samples were thawed once for portioning of aliquots and once for the laboratory analysis. Retinol and carotenoids were analyzed by HPLC; retinol by a modification (26) of a standard method (27) and carotenoids by a modification (26) of a new method (28).

Statistical analyses were conducted using SYSTAT (version 3.0, Systat, 1985) and the supplementary logit program on an AT&T 6300 Personal Computer. Differences in means were tested with the Student's T-test and differences in proportions were tested using logit analysis. In the latter analyses, case status was the independent variable, the breast cancer risk factor was the dependent variable and other breast cancer risk factors were added to the model as needed. Nutrients were log-transformed and categorized into quartiles based on their distribution in the

controls. Multivariate analyses used logit analysis with case status as the dependent variable; there were three possible outcome categories but only the case versus control results are presented here. In the multivariate analyses, the non-nutrient breast cancer risk factors used as covariates were age, age at menarche, age at menopause, age at first birth, parity, menopausal status, family history of breast cancer in a first-degree relative, Quetelet index (kg/m^2), marital status and income. Analyses of the plasma retinol and carotenoid variables also controlled for plasma lipids to evaluate the effect of the nutrient of interest after differences in lipid status were eliminated. In addition, both triglycerides and cholesterol were associated with case status and therefore were potential confounding variables. Tests for trend used the continuous, log-transformed nutrient variable. The models and variables were assessed using the log likelihood ratio test and all significant predictors of case status were included in the full models, as well as the non-nutrient breast cancer risk factors.

Results

The characteristics of the cases and controls at baseline are presented in Table 1. Cases were statistically significantly older than controls, but were not statistically different from controls in any of the other potential confounding variables. Nonetheless, many of the known breast cancer risk factors were not more prevalent among cases than controls. Therefore, these controls were at high risk for breast cancer according to known breast cancer risk factors. Thus, this study compared

women with breast cancer with a group who had similarly high risk, but who did not have breast cancer.

Cases had significantly lower beta-carotene and lycopene values than controls ($p<0.01$ and $p<0.05$, respectively) (Table 2), but were not different in the mean values for retinol, alpha-carotene or the dietary estimates of vitamin A intake. Multivariate analysis showed that low beta-carotene was associated with an increased risk of breast cancer, with and without adjustment for plasma lipids (Table 3). Alpha-carotene was not associated with breast cancer risk, perhaps because of its low concentration and small range (Table 2), making it difficult to assess its effect given the sample size in this study. In addition, the collinearity between beta-carotene and alpha-carotene (correlation coefficient = 0.71) made it difficult, if not impossible, to assess the independent alpha-carotene effect when the full model required both carotenoid variables. Although low levels of lycopene appeared to be associated with increased risk of breast cancer before controlling for plasma lipids, lycopene was not associated with case status after controlling for these lipids.

The main effect of plasma retinol values indicated no association with breast cancer risk (Table 3); however, a statistically significant interaction with plasma beta-carotene (Table 4) showed a trend of increasing risk for breast cancer with increasing retinol within the low beta-carotene quartile. The wide confidence intervals indicated few individuals in the cells with increased risk; even so, the results were statistically significant. Although the other confidence intervals included unity, the trends indicated that, within the other beta-carotene quartiles, risk decreased as retinol increased.

Multivariate analyses of the dietary data indicated no association between breast cancer and either estimate of vitamin A intake (Table 5). There was no statistically significant interaction between total vitamin A and vegetable vitamin A. Neither of the dietary variables was correlated with plasma retinol, beta-carotene or lycopene values. Alpha-carotene, however, was marginally correlated with total vitamin A ($r = 0.26$).

Discussion

The Control Group

The control group in this study were women at high-risk for breast cancer. There are two possible biases of concern with this control group. If factors associated with the high-risk classification were also associated with nutrient status, then the results may be biased towards a false positive or spurious finding. No evidence was found supporting an association between benign breast disease and any of the nutrients investigated, and it is unlikely that other components of the high-risk classification (i.e., age at menarche, age at first birth, etc.) are related to these nutrients. In contrast, the controls were very similar to the cases, which would bias the results towards a false negative finding of no association. This is only of concern with the nonsignificant alpha-carotene and lycopene findings but is not of concern with the significant beta-carotene and retinol findings. Indeed, the similarity between the groups may be a strength of the study because the absence of unnecessary variability may have increased the power to observe associations. That is, the analysis focused on the factors that allowed the controls to be free of cancer even though they were very similar to

the cases and at high-risk of breast cancer. A difference in nutritional status between these two groups would be of considerable interest because it would suggest a nutritional advantage among the controls very late in the promotional stages of the disease process. These results not only indicated such a difference existed between these groups but also that the difference was in the same direction (low beta-carotene, higher risk) as that frequently observed in earlier etiological stages.

Plasma Beta-carotene

The levels of carotenoids in this study were relatively low compared to similar groups of women in the United States (29,30). Quality control plasma samples were exchanged between laboratories, which determined that our assay yielded values approximately one-half the values obtained by other standard methods (31,32) due to lower extraction efficiency of the solvent. All carotene values are reported here in the original form because relative, not absolute values are required for the statistical analyses.

The association of lower beta-carotene values with breast cancer may be confounded by alcohol intake, which was not measured in this study. Alcohol has been associated with breast cancer in 14 studies (33-46), but not in 7 other studies (47-53). In addition, alcohol may be associated with lower plasma beta-carotene values (30, 54), although this effect has only been observed in men and it is unclear whether alcohol is directly related to lowering beta-carotene or is coincident with lower beta-carotene intake. Further research is warranted to determine whether the

association of plasma beta-carotene values with breast cancer persist after controlling for alcohol consumption.

Lower plasma beta-carotene values in the breast cancer patients may also be a result rather than a precursor of disease. A marginally significant trend ($p=0.08$) of decreasing beta-carotene values with increasing stage of disease was observed; this suggests that the disease may alter this blood parameter. Alternatively, the patients with later stage disease may have altered their intake to a greater degree than the patients with earlier stage disease. Although it is unclear which phenomenon is present, there is some evidence against a metabolic alteration due to the disease. A prospective study in Britain (18) demonstrated that the cases diagnosed within two years of blood collection did not have lower beta-carotene than cases diagnosed later. In addition, there is no evidence from animal studies that tumors affect the levels of this nutrient, whereas there is evidence that low beta-carotene intake promotes mammary tumors (10).

Plasma beta-carotene has been shown to reflect dietary intake of beta-carotene (24,55,56) and total carotene intake (30,54,57), therefore, the lower beta-carotene levels observed to be associated with case status may be of dietary origin. Although the dietary data from this study did not support this conclusion, results from larger dietary studies indicated that lower estimated intakes of total vitamin A (21) or beta-carotene (17) were associated with breast cancer.

There have been four other investigations of breast cancer that have measured plasma carotene values. In a prospective study in England (18), the authors found (nonsignificantly) lower plasma beta-carotene values in

women later diagnosed with breast cancer. Although these findings were later questioned because of storage considerations (58), the present study corroborates these findings. In a preliminary report (24), the mean plasma beta-carotene level of 30 women with metastatic breast cancer was 21% lower than in controls. Willett and coworkers (19) did not find an association between breast cancer and plasma carotenoids; this study was limited by sample size (n=14 cases) and did not control for important confounding factors, however. In a case-control study in Italy (16), the authors did not find an association between breast cancer and plasma beta-carotene values. The mean beta-carotene values were relatively high in this population, which suggests that there were few individuals in the low range where the effect was observed in the present study (even if our values were doubled to allow for analytical extraction efficiency).

Plasma Retinol

The higher risk observed with higher retinol within the low beta-carotene quartile is consistent with the concept that diets with higher intakes of animal products and lower intakes of vegetables may increase breast cancer risk. These results are supported by studies that associated breast cancer with low dietary intake of total vitamin A (21, 22) or beta-carotene (17) and high plasma retinol (16). Rohan and coworkers (17) also reported significantly increased risk of breast cancer with increased consumption of retinol, although the results were confined to premenopausal cases. The strongest univariate correlations for plasma retinol from a large ecological study in China (59) show that it is positively correlated to meat and milk consumption (r= 0.44 and 0.31,

$p<0.001$ and $p<0.050$, respectively) and negatively correlated with plant protein and legumes ($r= -0.50$ and -0.31, $p<0.001$ and $p<0.050$, respectively). However, there are several reports of no association between dietary vitamin A and plasma retinol (24,55-57,60). Additionally, retinol has been positively associated with plasma estrogens (61) and, because estrogens may be associated with breast cancer promotion, higher retinol levels may merely be a marker for the estrogenic environment or, perhaps, part of the mechanism by which estrogens exert their effect. Nonetheless, the pattern of risk observed suggests that beta-carotene is not acting solely through its conversion to retinol, and that these two nutrients may have some interdependent functions in carcinogenesis.

Dietary Data

The results from the dietary data were consistent with one study (16) that did not find an association between breast cancer and dietary beta-carotene. Other dietary studies (17,21,22) with larger sample sizes (451, 2024 and 120 cases, respectively) than the present study did show associations using dietary indicators of vitamin A or beta-carotene.

Preliminary analysis of the reproducibility of the dietary data suggests poor agreement in frequency categories between first and second questionnaires (59% exact agreement, 71% agreement plus or minus one frequency category). Thus, these dietary data included a large amount of unaccounted for variability, which biased the results toward the null hypothesis. The positive findings using the plasma indicators, the lack of a correlation between the plasma and dietary indicators of vitamin A and the lack of reproducibility of the dietary data suggest that dietary

indicators were not as sensitive as plasma indicators. Therefore, a larger samples size would be required for the food frequency methodology employed here to be useful.

Commentary

There were two other results of methodologic importance. First, the lack of a main effect of plasma retinol and the presence of an interactive effect between plasma retinol and beta-carotene demonstrated the importance of investigating nutritional interactions in studies on cancer etiology. Second, plasma lycopene values were associated with case status only before the plasma lipids were controlled in the analysis, which demonstrated the importance of this adjustment in the analyses of lipid-soluble nutrients. It was necessary to control for the these lipids to observe the independent effect of the nutrient (or lack of an effect) after differences in lipid-status were eliminated and the association of the lipid with case status was removed. Stryker et al. (30) demonstrated a better correlation between calorie-adjusted dietary intake of fat-soluble nutrients (carotenoids and tocopherols) and the lipid-adjusted plasma levels of these nutrients compared with the unadjusted levels. Therefore, the inference of risk estimates from plasma data to dietary intake is stronger after the plasma variables have been adjusted for these lipids.

The beta-carotene findings in our study are in accord with studies finding an association between low beta-carotene and cancers of epithelial origin (62). It is important to note, however, that high plasma beta-carotene may also be a marker for carotene-rich foods so that other

carotenes, or other constituents of these foods, may be the more important risk factors. Low plasma beta-carotene in combination with high retinol was found to be associated with breast cancer, which was consistent with diets lower in carotene-containing vegetables and higher in preformed vitamin A. Further research into this interaction by other investigators is recommended.

Table 1

Descriptive statistics of potential confounding variables for cases and controls, breast cancer study

Variable	Cases Mean (SD)[a] (N=83)	Controls Mean (SD) (N=113)
Age (yr)	57.78 (11.01)*	50.47 (10.81)
Weight (kg)	69.79 (13.49)	67.85 (12.00)
Height (cm)	164.75 (7.33)	165.19 (7.93)
Quetelet index (kg/m^2)	25.82 (5.16)	24.89 (4.52)
Age at Menarche (yr)	12.83 (1.30)	12.70 (1.57)
Age at First Birth (yr)	23.43 (3.95)	23.37 (4.29)
Parity	3.03 (1.36)	3.36 (1.63)
Age at Menopause (yr)	47.66 (6.27)	47.02 (5.92)
Months of Breastfeeding[b]	5.21 (9.01)	6.70 (10.47)
	(% of diagnostic group)	
Menopausal	66	57
Surgical Menopause[c]	40	33
Family History[d]	28	39
Married	71	87
Never Married	13	9
Widowed	16	4
Nulliparous	16	17

History of BBD[e]	29	40
Two Highest Income Groups[f]	29	42

a Abbreviations used; BBD = benign breast disease, SD = standard deviation.

b Total months in lifetime spent breastfeeding.

c Percent of those who were menopausal.

d Family history of breast cancer

e Self-reported history of a previous diagnosis of BBD.

f Two highest of 7 income categories

* Significantly different ($p<0.001$) from controls.

Table 2

Comparison of plasma and dietary variables

among cases and controls, breast cancer study

Variable	Cases (N=81)	Controls (N=101)
Plasma:		
Retinol (mg/dl)	55.98 (17.72)[a]	55.23 (12.66)
Beta-carotene (ug/dl)	11.20 (8.62)	13.75 (8.02)**
Alpha-carotene (ug/dl)	3.06 (2.18)	3.44 (1.99)
Lycopene (ug/dl)	22.36 (10.11)	26.38 (12.76)*
Diet:		
Total Vitamin A (IU/day)	11,900 (6,800)	12,300 (6,800)
Vegetable Vitamin A (IU/day)	1,770 (2,400)	1,900 (2,900)

a Mean (standard deviation)

** Significantly ($p<0.01$) different from cases

* Significantly ($p<0.05$) different from cases

Table 3

Case versus control odds ratios for plasma nutrient quartiles with adjustments for potential confounding variables[a] with and without lipid adjustment[b], breast cancer study

	Quartiles				
	Low			High	Trend
Nutrient	Q1	Q2	Q3	Q4	p-value
Lipid-unadjusted:					
Beta-carotene	4.42[c]	1.90	1.41	1.00	<0.01
	(1.33,14.7)[d]	(0.55,6.55)	(0.43,4.64)		
Alpha-carotene	2.69	1.12	0.96	1.00	0.18
	(0.88,8.23)	(0.36,3.51)	(0.31,2.99)		
Lycopene	2.40	2.45	1.55	1.00	0.09
	(0.83,6.95)	(0.87,6.93)	(0.51,4.72)		
Retinol	1.11	1.21	0.64	1.00	0.67
	(0.38,3.26)	(0.41,3.55)	(0.23,1.83)		
Lipid-adjusted:					
Beta-carotene	3.15	1.79	1.18	1.00	0.02
	(0.90,11.04)	(0.50,6.39)	(0.34,4.09)		
Alpha-carotene[e]	0.63	0.45	0.61	1.00	0.19
	(0.13,2.95)	(0.11,1.80)	(0.18,2.06)		
Lycopene	1.60	1.94	1.39	1.00	0.43
	(0.50,5.18)	(0.64,5.88)	(0.45,4.32)		
Retinol[e]	1.06	1.01	0.60	1.00	0.41
	(0.31,3.64)	(0.32,3.25)	(0.18,1.97)		

a Adjusted for age, age at first birth, family history, age at menarche, Quetelet index, parity, age at menopause, income and marital status.

b Lipids include plasma cholesterol and triglyceride

c Odds ratio of quartile compared with the fourth quartile

d 95% confidence limits

e Full model also contained beta-carotene

Table 4

Case-control adjusted[a] odds ratios for the retinol by beta-carotene interaction, breast cancer study

Retinol Level (percentile)[b]	Odds Ratios (CI) Beta-carotene Quartiles Low Q1	Q2	Q3	High Q4
Low[c] (25)	3.41 (0.75,15.6) [9/7][d]	4.07 (0.90,18.5) [5/5]	3.64 (0.66,20.1) [5/3]	3.85 (0.84,17.6) [4/4]
Median (50)	5.50 (1.30,23.2) [14/14]	2.90 (0.71,11.9) [9/14]	1.92 (0.45,8.21) [9/14]	1.92 (0.92,4.00) [4/10]
High (75)	8.58 (1.82,40.5) [12/4]	2.11 (0.48,9.30) [3/6]	1.06 (0.23,4.91) [4/8]	1.00[e] [3/8]

a Adjusted for cholesterol, triglyceride, beta-carotene, the retinol by beta-carotene interaction, age, age at first birth, family history, age at menarche, Quetelet index, parity, age at menopause, income and marital status.

b Percentile based on ranking of all study subjects

c Low=43.6 ug/dl, Median=52.6 ug/dl, High=62.7 ug/dl

d Number cases/controls

e Reference group

Table 5

Case versus control odds ratios for dietary nutrient quartiles with adjustments for potential confounding variables in the full model[a], breast cancer study

	Quartiles				
	Low			High	Trend
Nutrient	Q1	Q2	Q3	Q4[b]	p-value
Total Vitamin A	1.35[c] (0.50,3.60)[d]	1.43 (0.53,3.87)	0.45 (0.14,1.45)	1.00	0.56
Vegetable Vitamin A	1.27 (0.44,3.67)	1.72 (0.66,4.48)	1.48 (0.50,4.36)	1.00	0.52

a Adjusted for age, age at first birth, family history, menarche, Quetelet index, parity, age at menopause, income and marital status.

b Reference group

c Odds ratio of quartile compared with the fourth quartile

d 95% confidence limits

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